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Electron Microscopy of a Rod-Shaped Noninclusion Virus Infecting the Citrus Red Mite[1,2]

D. K. REED

Entomology Research Division, Agricultural Research Service U. S. Department of Agriculture, Riverside, California 92502

AND I. M. HALL

Department of Biological Control, University of California, Riverside, California 92502

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Thin sections of the citrus red mite, *Panonychus citri*, infected with a virus disease contained rod-shaped virions but no rods were found in healthy mites. The rods, which were present in midgut epithelial cells, were ca. 194 × 58 nm and were enclosed in an envelope ca. 266 × 111 nm. Some mites, both healthy and diseased, contained spherical particles, but these particles did not cause symptoms of the virus disease.

INTRODUCTION

A disease affecting the citrus red mite, *Panonychus citri*, was reported by Munger et al. (1959) and shown subsequently (Gilmore, 1965; Shaw et al., 1968) to be important in the natural control of this pest. The viral nature of this disease was first suspected by Smith et al. (1959). Those investigators were not able to obtain ultrathin sections, but they did isolate spherical particles (icosahedrons ca. 35 nm in diameter) by differential centrifugation. Therefore, since noninclusion viruses infecting arthropods are usually spherical or near-spherical, the citrus red mite virus has since been considered to have this shape. However, Bird (1967) reported finding rod-shaped virions in sections of the nuclei of fat cells of *Panonychus ulmi* showing symptoms of a similar disease (reported by Steinhaus, 1959), that had also been presumed to have spherical-to-ellipsoidal viral particles. Thus the viral particles causing the disease of *P. citri* might still be unknown. A new investigation of the disease was therefore initiated.

MATERIALS AND METHODS

The citrus red mites used in the study were obtained from a colony maintained at the Arid Areas Citrus Insects Investigations Laboratory at Riverside, California. The mites were reared on green lemons at 25°C and 60% RH (Munger and Gilmore, 1963) and the life cycle from egg to adult was completed in about 14 days. Inoculation of the mites with the virus was accomplished in one of two ways. By one method, green lemons infested with deutonymphs were sprayed with aqueous suspensions of triturated diseased mites; these sprays were applied with an atomizer at a concentration of 1 mg/ml and a volume of 0.5 ml/lemon. By the other method, young adult mites were fed the same material mixed in a 10% sucrose solution through a duPont polyethylene 100 film for 4–6 hr; then they were transferred to green lemons with a small brush. Control mites were either untreated or fed 10% sucrose. The treated and control mites were maintained in a cloth-covered cabinet at the temperature and humidity of

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the rearing room until they were removed for examination.

Virus-treated and control mites were fixed for 1 hr in glutaraldehyde buffered at *p*H 7.0 with 0.1 *M* potassium phosphate buffer. (Most mites were pierced with a fine needle before fixation to increase the penetration.) Then they were washed in buffer, postfixed with OsO_4 for 1 hr, and dehydrated through a graded series of ETOH. However, the cuticle of the mite is hydrophobic so a drop of Kodak Photo-Flo was added to the fixatives to facilitate immersion. In the early part of the work the mites were embedded in Maraglas (Freeman and Spurlock, 1962), and in the later part Spurrs' Epoxy resin medium (Spurr, 1969).

The prepared mites were oriented to proper positions within the blocks, and sections (50–100 nm thick) were cut with a diamond knife on a Porter-Blum MT-1 or MT-2 ultramicrotome. These sections were mounted on bare 300- or 400-mesh copper grids or on Formvar-coated 200-mesh grids, stained with 1% aqueous uranyl acetate for 1 hr, followed by lead citrate (Reynolds, 1963) for 5 min at room temperature, and examined with a Philips 300 electron microscope operated at an accelerating voltage of 80 kV.

For the light microscope studies that were necessary to determine the location of cells observed with the electron microscope, mites were fixed in glutaraldehyde or paraformaldehyde and embedded in glycol methacrylate (Feder and O'Brien, 1968). Then 1 -to 3-μ sections were cut with glass knives and stained with Mallory-Heidenhain stain (Cason, 1950).

Results and Discussion

A section of a healthy citrus red mite female under the light microscope is shown in Fig. 1. The midgut occupies practically all the dorsal and lateral parts of the body cavity, except for that occupied by the hindgut. The midgut epithelium is composed of glandular cells of variable size and shape with typically large nuclei. Free cells that apparently slough off from the epithelium are usually seen within the lumen of the gut. Hindgut cells have a pe-

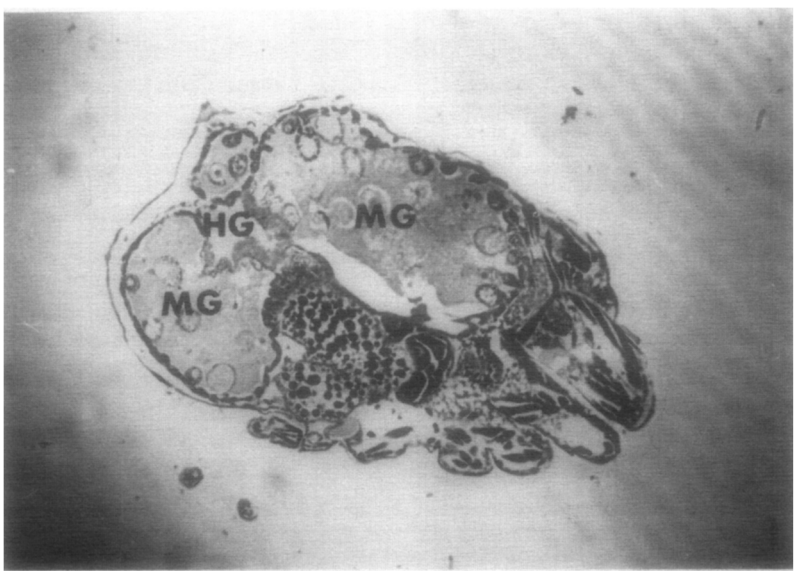


Fig. 1. Longitudinal section through a healthy adult female *Panonychus citri*. Midgut (MG) surrounds the hindgut (HG). ×210.

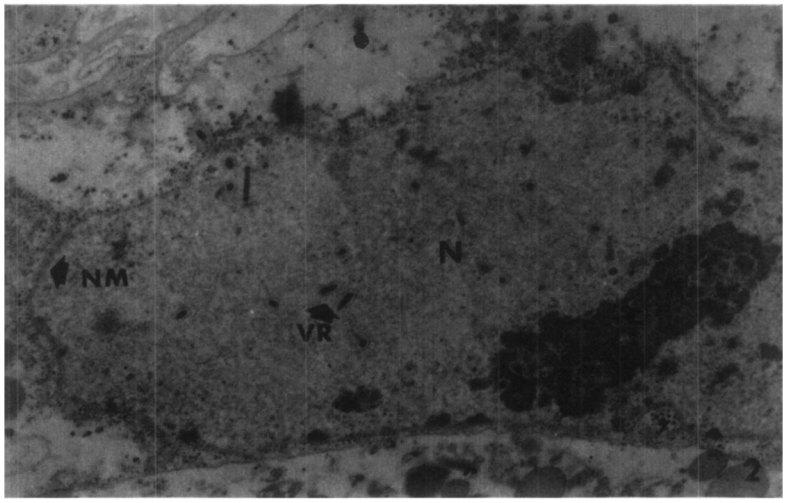
culiar vesiculate appearance, and the organ is surrounded for most of its length by the ventriculus. Through the light microscope, we perceived no changes in either the midgut or hindgut cells of diseased mites.

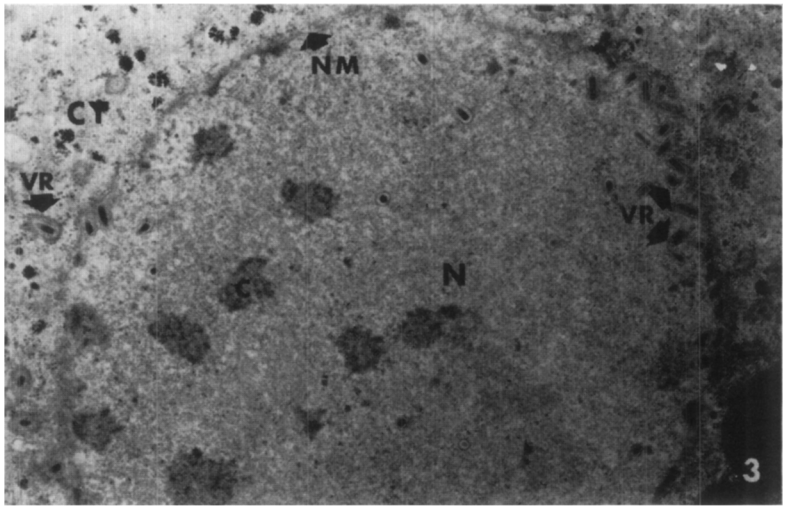
The examination of the thin sections with the electron microscope revealed that in diseased mites rod-shaped virus particles were abundant in nuclei of midgut epithelial cells. Although Bird (1967) reported finding rods from fat cells of *P. ulmi*, no virus rods were identified from such tissue in *P. citri*, although most of our observations were made of the posterior part of the mite. Also, when we examined mites fed 6 hr on the virus-sucrose mixture and then transferred to a green lemon for 18 hr (thus a total elapsed time of 24 hr postinoculum before they were fixed and examined), we found rod-shaped virus particles in the nuclei of midgut epithelial cells; however, no rods were observed in mites fixed 18 hr after exposure. Thus the virions obviously require more than 18 hr to develop. Healthy control mites were always free of the rods, but virus rods were found consistently in diseased mites up to and including those that died from the disease, usually at about 9 days after spraying or 5–6 days after feeding.

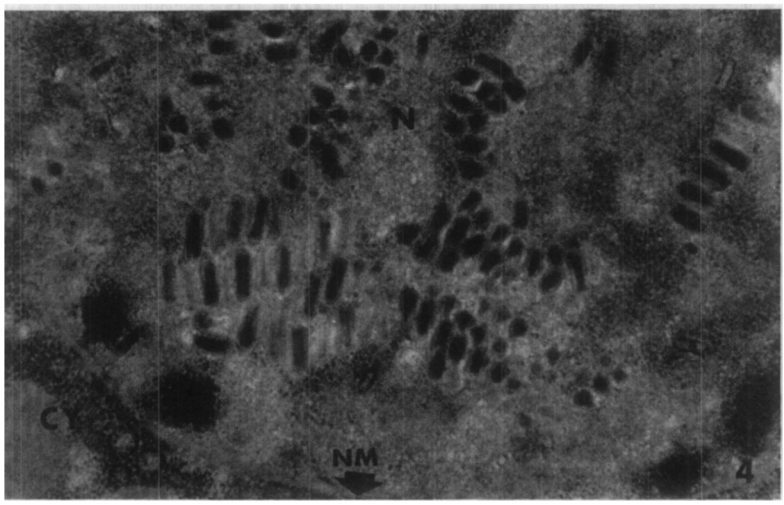
Portions of typical midgut epithelial cells from diseased mites are shown in Figs. 2–4. At 24 hr after inoculation, the rods have begun to form and are moving out into the cytoplasm (Fig. 2). At 48 hr, many rods have gathered against the nuclear membrane and many are in the cytoplasm (Fig. 3). Figure 4 shows the nucleus 10 days after the mites were sprayed with inoculum when many more epithelial cells contained rods. The virus particles within the nucleus consisted of an electron-dense core (194.2 $\pm$ 16.3 $\times$ 58.3 $\pm$ 7.2 nm) surrounded by a clear area which was enclosed within a single membrane 265.9 $\pm$ 28.6 $\times$ 110.8 $\pm$ 11.45 nm. Measurements were obtained from contact prints with an optical vernier device and therefore should indicate the general range of size though they may not be completely accurate since some obliquely sectioned rods may have been measured. No structure was observed in the core. Considerably chromatinlike material was present in early stages of the disease (Figs. 2, 3) but not in later stages, and it has not been determined if it was involved in virus replication. All rods observed in our preparations were nearly identical in appearance, though somewhat larger, to those described by Bird (1967) from the European red mite (200 $\times$ 90 nm overall with a 150 $\times$ 38 nm core).

All rods possess a single membrane. In the nucleus this is the only membrane around each rod. Such structure is very evident in Fig. 5. However, as the virions pass out into the cytoplasm in a budding manner (Fig. 6), they acquire an additional outer double membrane originating from nuclear membrane material. Rods may either be singly or multiply enclosed by the membrane depending on the number that budded through at a certain point. Both single and multiple enclosed rods may be seen within the cytoplasm of the cell shown in Fig. 5.

Since the early investigators of the virus of *P. citri* found spherical bodies, we searched for spheres in our material. In the present study, they were found in only 2 of 19 diseased mites and 4 of 10 healthy mites. All 6 mites had been fed sucrose. In healthy mites, the spheres were ca. 24–25 nm in diameter and in a crystalline array within the cytoplasm of epithelial cells (Fig. 7). One diseased mite had spheres of this size in a crystalline array, but the other had many spheres, ca. 34–35 nm in diameter, in the cytoplasm of an unidentified cell. Since spheres in this size range were isolated from diseased mites by Smith et al. (1959) and were subsequently isolated in great numbers in another study at the Boyden Laboratory from both diseased and healthy mites (unpublished), they probably are abundant only in tissues that were not examined in

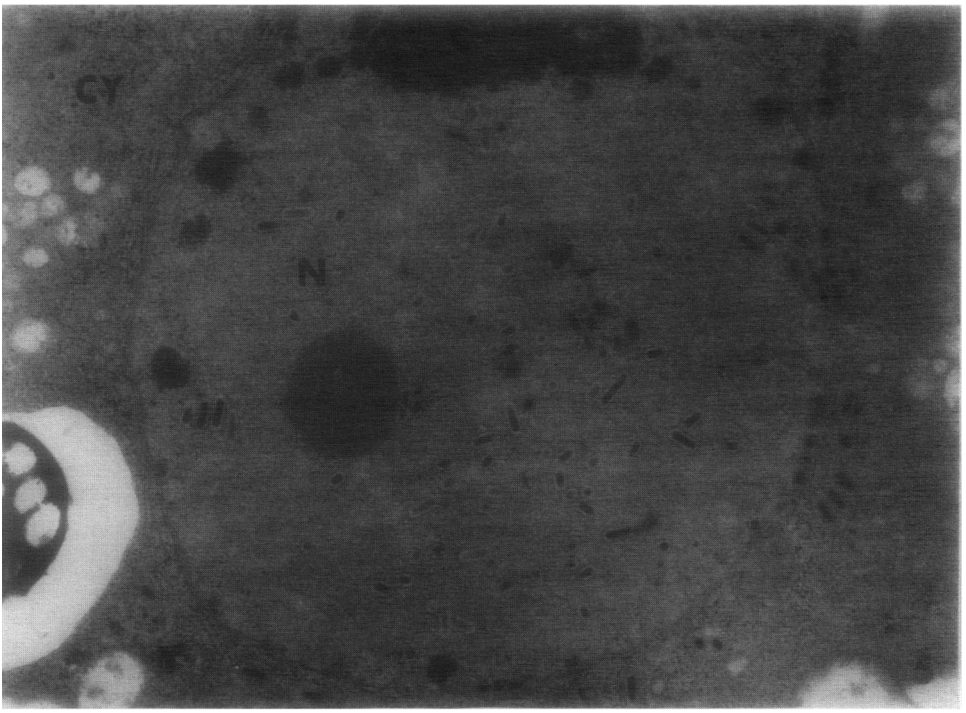


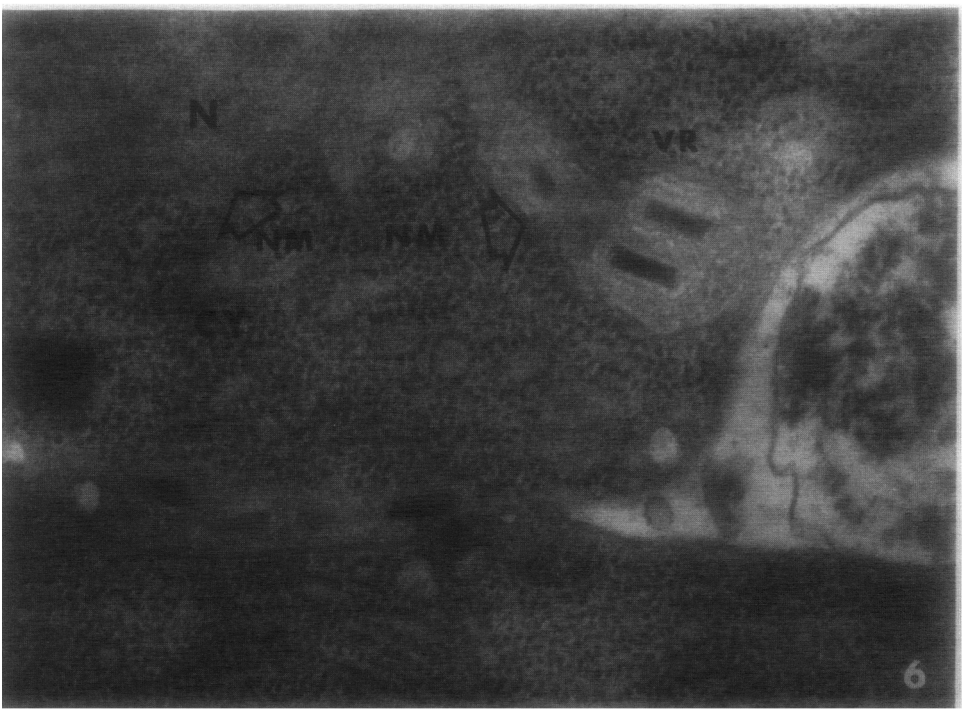




FIGS. 2 and 3. Ultrathin sections through midgut epithelial cells of *Panonychus citri* at 24 hr (Fig. 2) and 48 hr post-inoculum (Fig. 3). Virus rods (VR) and chromatinlike material (c) are present in the nucleus (N). Rods are being budded off through the nuclear membrane (NM) into the cytoplasm (Cy) in both figures. ×16,400.

FIG. 4. Section through midgut cell of moribund diseased mite showing great numbers of virus rods, many within a regular array. ×35,100.





FIGS. 5 and 6. Sections showing the presence of rods within the nucleus and also being budded out into the cytoplasm. Notice the nuclear membrane around those rods in the cytoplasm in Fig. 5. ×20,000. In Fig. 6 (×70,700), the mechanism of budding is clearly shown.

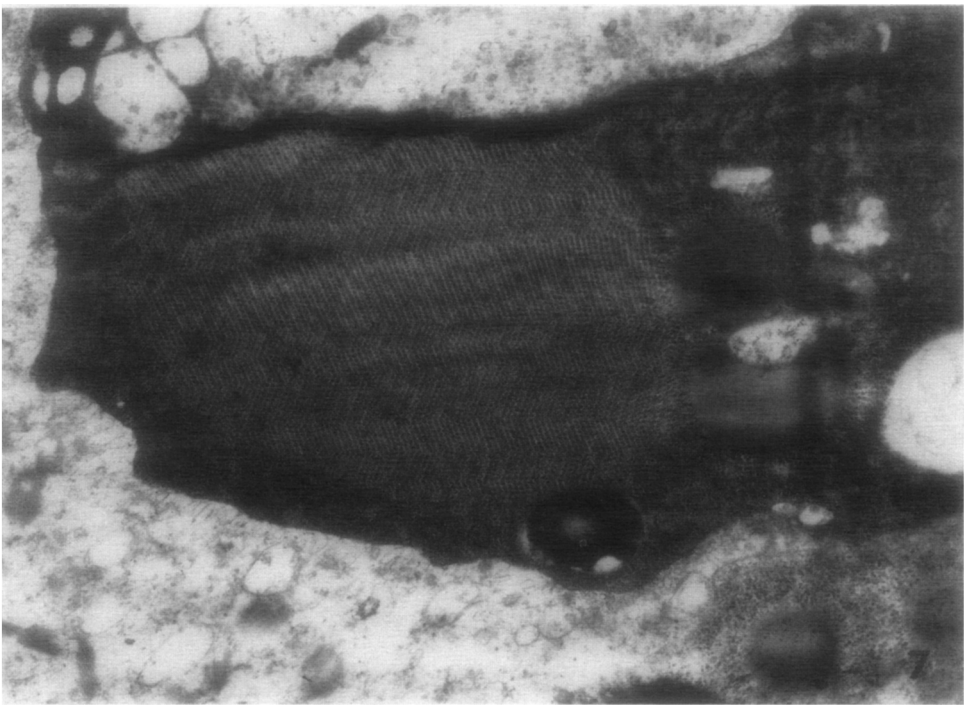


FIG. 7. Section of a healthy mite through a midgut epithelium cell which has broken off from the epithelium and is floating free in the midgut lumen. A crystalline array of small spheres is present within the cytoplasm. ×20,300.

detail in the present study. Also, when we bioassayed the spheres against healthy mites, they did not produce the typical symptoms of the disease (unpublished).

Hüger (1966) described a virus affecting an Indian rhinoceros beetle, *Oryctes rhinoceros*, in which both nonoccluded rods and spheres were present within the nuclei of fat cells. We did not find spheres within the same cells as the rods so the two viruses are undoubtedly different, but the similarity in size of nonoccluded rods in the rhinoceros beetle and in the citrus red mite may indicate a relationship between the two viruses, especially since nonoccluded rod-shaped virions are apparently a rarity in invertebrate hosts.

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